1-1-2002

Evaluating under-used conifers in Iowa

Carol Diane LaFaver
Iowa State University

Evaluating under-used conifers in Iowa

by

Carol Diane LaFaver

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Horticulture

Program of Study Committee:
Jeffery K. Iles, Co-major Professor
Janette R. Thompson, Co-major Professor
Mark L. Gleason
William R. Graves

Iowa State University

Ames, Iowa

2002

Graduate College
Iowa State University

This is to certify that the master's thesis of

Carol Diane LaFaver

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy

Table of Contents

Abstract

Moisture extremes, insect and nematode pests, and fungal pathogens have combined to limit the range of conifer species used in amenity plantings and windbreaks in Iowa and much of the Midwest. Responding to the need for a broader conifer palette, we attempted to identify species for increased use in Iowa. One study was done to determine a media for optimal mycelium and conidia production of *Dothistroma pini* Hulbary, and two separate studies were conducted to characterize landscape suitability of some under-used conifer species.

The first study evaluated 5 different media types for growing *Dothistroma pini* Hulbary in culture. We hypothesized a low-nutrient agar would result in poor mycelium growth and enhanced conidia production. Five different media were used and 5-mm plugs of *D. pini* were transferred to plates under sterile conditions. Colony diameter was measured every five days and conidia were harvested and counted in a hemacytometer under a microscope. Contrary to previous research, potato dextrose agar (a high-nutrient media) produced significantly more mycelium and conidia than more nutrient deficient media.

The second study involved determining the susceptibility of *Pinus leucodermis* Antoine (Bosnian pine) and *Pinus koraiensis* Siebold & Zuccarini (Korean pine) to *Dothistroma pini* (Dothistroma needle blight). *Pinus nigra* Arnold (Austrian pine), regarded as highly susceptible to *D. pini*, was used as the comparison species. Container-grown plants were inoculated with 15 ml of conidial suspension and placed under optimum conditions for infection in a growth chamber. After 24 hours, plants were placed in the greenhouse and observed twice weekly for infection incidence and infection severity for 90 days. We also

measured growth (height and stem diameter) and several physiological parameters (dry weights of plant tissues, photosynthetic rates at days 45 and 90, and starch storage in root tissues). *P. leucodermis* and *P. koraiensis* were susceptible to *D. pini* to the same extent as *P. nigra*. There were no differences among species in infection incidence and infection severity.

Finally, an outplanting study was conducted to evaluate survival and growth of *Abies homolepis* Siebold and Zuccarini (Nikko fir) in central Iowa. In June 2000, 148 *A. homolepis* and 133 *Picea abies* (L.) Karstens (Norway spruce) were planted at an ISU Forestry research site in Ames, Iowa. Over an 18-month period data were collected on survival, height, diameter, new growth, and lowest lateral length were measured. Soil samples were taken across a transect of the plot and analyzed for particle size distribution. No differences between species were found in growth or survival. Survival of trees was affected by location in the field plot.

Since *P. leucodermis* and *P. koraiensis* are susceptible to *D. pini*, these taxa may be suitable for planting only on a limited basis. *A. homolepis* requires screening for pest and pathogen susceptibility and may be site-sensitive, but shows potential as a specimen plant for managed landscapes.

Chapter 1. General Introduction

Introduction

From managed urban landscapes to windbreaks and Christmas tree plantations, conifers have long been important landscape components. In Iowa, *Pinus strobus* L. (Eastern white pine) and *Juniperus virginiana* L. (Eastern red cedar) are the only widespread native conifers. Extreme climatic conditions in combination with pest and pathogen pressures have limited our conifer choices. Commercial producers and consumers alike are demanding more plant material options; unfortunately, overlooked or under-used conifer species may be difficult to locate in the nursery trade.

This study was originally conceived to answer the question, where do we look to find new conifers suited for the Iowa landscape? Between September 1998 and June 1999 we visited specialty conifer collections at arboreta and private gardens around the Midwest. During these travels, 56 species of *Pinus*, *Picea*, and *Abies* were cataloged and evaluated as potential subjects for inclusion in our research. Eventually, two pines and one fir were identified for further investigation. Our overall objective was to identify species that could withstand climatic stress and that would be less susceptible to *Dothistroma pini* Hulbary currently plaguing conifers in the Upper Midwest.

Thesis Organization

This thesis consists of three manuscripts. The first manuscript, chapter 2, will be submitted to *Plant Health Progress* and is formatted for that online journal. This paper describes a comparison of media used to grow *Dothistroma pini* Hulbary in culture. The second manuscript, chapter 3, is formatted for the *Journal of Environmental Horticulture*. This manuscript describes an inoculation study that determined susceptibility of *Pinus* *leucodermis* Ant. and *Pinus koraiensis* Sie. & Zucc. to *Dothistroma pini H.*. And finally, the third manuscript, chapter 4, has been formatted for submission to *Landscape Plant News*. This final paper is a field study examining Nikko fir planted in central Iowa. A literature review and general conclusions for this research are included.

Literature Review

Dothistroma pini Hulbary, commonly known as red band needle blight or Dothistroma needle blight, is an important and destructive fungal pathogen of many species including *Pinus nigra* (Austrian pine) and *Pinus ponderosa* (ponderosa pines). The disease is widespread throughout the United States in both managed landscapes and reduced-maintenance green spaces. *Dothistroma pini* H. was first recognized by Robert Hulbary as a foliar fungus blight infecting Austrian pine in Illinois. Prior to his discovery that it was indeed a fungus, decline of Austrian pine was attributed to a variety of environmental factors, alone or in combination with other abiotic and biotic factors such as insects (Hulbary, 1941). After dissecting infected needles, however, he discovered a fungus was responsible for this disorder on pine and named the new pathogen *Dothistroma pini* (Hulbary, 1941). Unfortunately, Hulbary failed to mention the appearance of red bands on needles as a symptom of *D. pini*.

Between 1954 and 1960, pines in Great Britain began dying from an unknown disease, characterized by red bands on the needles. Only later was this symptom officially attributed to *D. pini* (Murray and Batko, 1962). Over the years, *Larix decidua* Miller (European larch), *Picea sitchensis* (Bong.) Carr. (Sitka spruce), and *Pseudotsuga menziesii* (Mirb.) Franco (Douglas fir), and approximately 20 species of pine have been identified as hosts for *D. pini* (Peterson, 1967a; Kershaw et al., 1982). *Dothistroma pini* is now known to occur in Australia, Europe, Asia, North America, South America, and Africa (Murray and Batko, 1962; Peterson, 1969; Edwards and Walker, 1978; and Kershaw et al., 1982). In the United States, 22 of the 50 states have reported pines infected by *D. pini* (Peterson, 1967a).

To date, *Dothistroma pini* has undergone many name changes. It was first placed in the genus *Septoria* where it remained until Hulbary's discovery in 1941 (Hulbary, 1941). The same fungus also was assigned to the genera *Actinothyrium, Cryptosporium, Hemidothis, Septocystis, Lecanosticta,* and *Tympanis* during the same time period (Hulbary, 1941; Thyr and Shaw, 1964). In 1941, Hulbary determined all of the above genera were the same fungus and thereby named *Dothistroma pini* as its own genus. In 1964, Thyr and Shaw proposed two varieties should be distinguished based on conidia length and width: *Dothistroma pini* Hulbary var. *pini* and *Dothistroma pini* Hulbary var. *linearis* (Thyr & Shaw, 1964).

D. pini is currently considered a major plant pathogen that causes widespread economic and aesthetic damage (Hansen and Lewis, 1997). The presence of this fungal disease worldwide is a classic example of exotic pathogens adapting and thriving in new environments (Hansen and Lewis, 1997).

Life cycle of *Dothistroma pini* H.

Like other Ascomycetes, *Dothistroma pini* has a perfect and imperfect stage in its lifecycle. The perfect, or sexual stage is spore-producing and extremely rare in nature; it is seldom considered a threat in the midwest United States (Funk and Parker, 1966; Peterson and Graham, 1974). The imperfect, or asexual, stage is conidia-producing and is most commonly found worldwide. In the central United States, conidia develop in fruiting bodies in the fall on infected needles and mature the following spring (Peterson, 1981). Mature conidia are spread by rainsplash from May to October (Peterson, 1981). Infection of a pine needle begins with the germination of conidia on the surface of the needle after which the germ tube invades the stomata (Peterson, 1967b). The stromata may develop enough to raise the needle epidermis (Peterson and Graham, 1974). Symptoms begin to appear from mid-September to December. The fungus overwinters on both infected needles that are still living and needles that have been shed due to fungal infection and on normal needle loss. Peterson and Harvey (1976) documented significant release of conidia with very small amounts (<0.25 mm) of precipitation. In warmer climates, this lifecycle can be completed in one growing season.

Typically, only needles from previous year's growth are infected; however, during years of high conidia production current years' needles can be severely infected. Conidia viability depends on position of the needle in the canopy and whether the stand has been thinned, pruned, or untreated (Gadgil, 1970). Conidia on infected needles suspended above ground in thinned stands remained viable for 4-6 months, whereas conidia on infected needles in the leaf litter were typically viable for only 2-4 months (Gadgil, 1970).

Methods of culturing

Culture techniques for *Dothistroma pini* H. have been studied to a much lesser degree than biology and control. Ivory (1967) reported conidia germination and growth was primarily dependent on media type. In that study, optimum colony growth occurred on 3% malt extract agar with trace amounts of nitrogen at pH 3.5 (Ivory, 1967). Later studies examined diameter growth of individual colonies as well as conidia production, and indicated that malt extract medium concentration affected number of conidia and amount of mycelium inversely (Rack and Butin, 1973). More recently, Bradshaw et al. (2000) suggested separate media should be used to culture mycelium and conidia.

Control

Studies in Nebraska showed that Bordeaux mixture or other copper-containing fungicides could provide good control of *D. pini* (Peterson, 1965; Peterson, 1967b). Early recommendations for chemical control of *D. pini* were based on Austrian pine phenology. An initial application of pesticides was suggested when needles were one-third elongated, and a second application when needles were two-thirds elongated (Peterson, 1965).

A second method of control is selection of resistant seed sources. Several *P. ponderosa* seed sources in the United States and one *P. nigra* seed source from Greece have been identified as potentially suitable for Midwest planting based on lower susceptibility to both first and second year needle infection (Peterson, 1984; Peterson and Read, 1971).

Tree Species

The tree species reported on in this thesis include three little-used exotic conifers, *Pinus leucodermis* (Bosnian pine), *Pinus koraiensis* (Korean pine), and *Abies homolepis* Siebold and Zuccarini (Nikko fir), and two conifers that are widely planted in Iowa, *Pinus nigra* (Austrian pine) and *Picea abies* (L.) Karstens (Norway spruce). Austrian pine has been used extensively in the Iowa landscape; however, it is no longer recommended by professionals due to its high susceptibility to *Dothistroma pini* and *Sphaeropsis sapinea* (Gleason et al., 2000). Norway spruce has long been planted in Iowa and continues to perform well in the landscape. Bosnian pine and Korean pine were identified as suitable candidates for study as additional pines that might be used in the landscape. Nikko fir was also identified as having potential for use in Iowa.

Bosnian pine is native to areas of Yugoslavia, Albania, Bulgaria, Greece, and Italy and is now a common ornamental pine to Europe (Krussman, 1985). Its characteristics are very similar to Austrian pine in terms of height, diameter, needles, and buds (Krussman, 1985).

Korean pine is a 5-needled pine native to Korea and mountainous areas of Japan (Dirr, 1990). It has a loose growth habit to a height of 40 feet. General observations conclude that it is very cold hardy with no serious pest or pathogen problems (Dirr, 1990). It is considered ideal for landscape use not only for its hardiness but also for its aesthetic qualities which include soft blue needles (Dirr, 1990; Krussman, 1985).

Nikko fir, native to Japan, is known for its highly aesthetic appeal in the landscape (Dirr, 1990). Nikko fir has been suggested to be highly tolerant of urban pollution and is reported to thrive under extreme conditions (Rushforth, 1987).

Physiology

Photosynthetic rates of trees with foliage diseases are typically reduced (Edmonds et al, 2000). Needle blights affect photosynthesis in a variety of ways including decreased photosynthetic area, reduction in amount of chlorophyll, interference from toxins released by the pathogen, and partial or complete stomatal closure (Agrios, 1988). Although photosynthetic rates may be unaffected in the initial stages of infection, they can decrease as much as one-fourth the original capacity after prolonged exposure to a pathogen (Heitefuss and Williams, 1976, Agrios, 1988). Although *D. pini* may not be virulent enough to kill a tree, by decreasing photosynthesis it can decrease tree growth and vigor, making the tree more susceptible to other pathogens, pests, or climate pressures (Edmonds et al., 2000).

The amount of plant carbohydrate storage, such as starch reserves in root tissue, can reveal a physiological response to pathogens. Starch stored in root tissues, typically amyloplasts, is mobilized as respiration increases in response to pathogen attack (Heitefuss and Williams, 1976; Agrios, 1988; Salisbury and Ross, 1992). Starch reserves maintained in root tissue thus are typically lower for plants affected by pathogens.

Literature Cited

Agrios, G.M. 1988. Plant Pathology. 3rd ed. Academic Press, San Diego, CA.

Bradshaw, R.E., R.J. Ganley, W.T. Jones, and P.S. Dyer. 2000. High levels of dothistromin toxin produced by the forest pathogen *Dothistroma pini*. Mycological Research 104: 325-332.

Dirr, M.A. 1990. Manual of Woody Landscape Plants. Their Identification, Ornamental Characteristics, Culture, Propagation, and Uses. 4th ed. Stipes, Champaign, IL.

Edmonds, R.L., J.K. Agee, and R. I. Gara. 2000. Forest Health and Protection. McGraw-Hill, Boston, MA.

Edwards, D.W. and J. Walker. 1978. Dothistroma needle blight in Australia. Australian Forest Research 8: 125-137.

Funk, A. and A.K. Parker. 1966. *Scirrhia pini* n. sp., the perfect state of *Dothistroma pini* Hulbary. Canadian Journal of Botany 44: 1171-1176.

Gadgil, P.D. 1970. Survival of *Dothistroma pini* on fallen needles of *Pinus radiata*. New Zealand Journal of Botany 8: 303-309.

Gleason, M.L., M. Linit, N. Zriba, P. Donald, N. Tisserat, and L. Giesler. 2000. Pine wilt: A fatal disease of exotic pines in the Midwest. Iowa State University Press. University Extension SUL-9, Ames, IA.

Hansen, E.M. and K.J. Lewis, eds. 1997. Compendium of Conifer Diseases. APS Press: St. Paul, MN.

Heitefuss, R and P.H. Williams eds. 1976. Physiological plant physiology (Encyclopedia of plant physiology; vol. 4). Springer-Verlag, New York, NY.

Hulbary, R.L. 1941. A needle blight of Austrian pine. Illinois Natural History Survey Bulletin 21: 231-236.

Ivory, M.H. 1967. Spore germination and growth in culture of *Dothistroma pini* var. *keniensis*. Transactions of the British Mycological Society 50: 563-572.

Kershaw, D.J., P.D. Gadgil, G.J. Leggat, J.W. Ray, and J.B. van der Pas. 1982. Assessment and control of Dothistroma needle blight. Revised Edition. FRI Bulletin No. 18, Rotorua, NZ.

Krussman, G. 1985. Manual of Cultivated Conifers. Timber Press, Portland, OR.

Murray, J.S. and S. Batko. 1962. *Dothistroma pini* Hulbary: A new disease on pine in Britain. Forestry 35: 57-65.

Peterson, G.W. 1965. Dothistroma needle blight of Austrian pine: Infection and control. Plant Disease Reporter 49: 124-126.

Peterson, G.W. 1967a. Dothistroma needle blight of pines in North America. Exposes XIV IUFRO Kongress: 269-278.

Peterson, G.W. 1967b. Dothistroma needle blight of Austrian and Ponderosa pines: Epidemiology and control. Phytopathology 57: 437-441.

Peterson, G.W. 1969. *Dothistroma pini* needle blight on *Pinus radiata*. FAO Plant Protection Bulletin 17: 43.

Peterson, G.W. 1981. Control of diplodia and dothistroma blights of pines in the urban environment. Journal of Arboriculture 7: 1-5.

Peterson, G.W. 1984. Resistance to *Dothistroma pini* within geographic seed sources of *Pinus ponderosa*. Phytopathology 74: 956-960.

Peterson, G.W. and D.A. Graham. 1974. Dothistroma needle blight of pines. U.S.D.A. Forest Service, Forest Pest Leaflet 143.

Peterson, G.W. and G.M. Harvey. 1976. Dispersal of *Scirrhia* (*Dothistroma*) *pini* conidia and disease development in a shore pine plantation in western Oregon. Plant Disease Reporter 60: 761-764.

Peterson, G.W. and R.A. Read. 1971. Resistance to *Dothistroma pini* within geographic sources of *Pinus nigra*. Phytopathology 61: 149-150.

Rack, K. and H. Butin. 1973. A quick method for the production of *Dothistroma pini* spores in culture. European Journal of Forest Pathology 3:201-209.

Rushforth, K.D. 1987. Conifers. Bath Press, Bromley, Kent, England.

Salisbury, F.B. and C.W. Ross. 1992. Plant physiology. 4th ed. Wadsworth publishing, Belmont, CA.

Thyr, B.D. and C.G. Shaw. 1964. Identity of the fungus causing red band disease on pines. Mycologia 56: 103-109.

Chapter 2. Testing five media types for growth and conidial formation of *Dothistroma pini* H.

A note to be submitted to *Plant Health Progress*

Carol D. La Faver, Mark L. Gleason, Janette R. Thompson, and Jeffery K. Iles

Abstract

Techniques for culturing of *Dothistroma pini* Hulbary have only been studied to a limited extent. Previous research has shown that high- and low-nutrient agars have inversely related effects on colony growth and conidia production with high nutrient agar producing low amounts of conidia. It has also been suggested that the presence or absence of light has no effect on colony size or conidia production. Our objectives were to determine what media could be used to produce the most mycelium and the most conidia and to determine if light had an effect on colony size and conidia production. Five media were used: water agar, water agar amended with pine needles, 2% malt extract agar, 1% malt extract agar, and potato dextrose agar. Five-mm plugs of *D. pini* mycelium were transferred to plates and placed in a growth chamber with either 16-hour light or continuous darkness. Colony size was measured every 5 days for 35 days and conidia were harvested and counted under a microscope at day 35. Potato dextrose agar produced the most mycelium and conidia. Light had a significant effect on colony size for 3 of the media tested (1% and 2% malt extract agars and potato dextrose agar) and on conidia for one of the media tested (potato dextrose agar).

Introduction

Dothistroma pini Hulbary is a forest pathogen which occurs in many countries and in 22 states in the United States. Since its discovery (Hulbary, 1941), *D. pini* has been found to infect a large number of ornamental pine species. However, only a limited number of studies have focused on culture techniques for conidia production by *D. pini*. In 1967, Ivory reported that spore germination and growth was primarily dependent on media type, proposing the addition of trace amounts of nitrogen and pH 3.5 being as optimal (Ivory, 1967). Ivory (1967) also suggested that light had no effect on colony size. Subsequent studies examined colony diameter growth and conidia production, noting the concentration of solution of malt extract medium affected the number of conidia and amount of mycelium produced (Rack and Butin, 1973). It was determined that a 2% malt extract agar at a pH of 5.5 was the optimum medium for conidia production of *D. pini* (Rack and Butin, 1973). Bradshaw et al. (2000) suggested different media for mycelium growth (5% malt extract, 2.3% nutrient agar) and conidia production (2% malt extract and 0.5% yeast extract). This research was based on a need for development of *D. pini* conidia in culture. This study utilized 5 different media for mycelium growth and conidia production of *D. pini* in culture. Our objectives were to determine which media produced the most mycelium and the most conidia, and to determine whether light had an effect on colony diameter and conidia production. Our hypothesis was that high-nutrient media would produce an abundance of mycelium and low concentrations of conidia, whereas low-nutrient media would produce less mycelium and more conidia. We also hypothesized that light would have no effect on colony size or number of conidia.

Materials and Methods

A *Dothistroma pini* Hulbary (Strain #26810) culture was obtained from American Type Culture Collection (Manassas, VA). Colonies were obtained by streak plating a conidial suspension on potato dextrose agar (4% w/v). All plates were 9 cm in diameter and 20ml of agar were added. Five-mm diameter plugs were transferred to water agar (1.5% (w/v) Difco-Bacto agar), water agar with one autoclaved *Pinus nigra* Arn. needle per plate (1.5% (w/v) Difco-Bacto agar), 1% malt extract agar (1% (w/v) malt extract, 1.5% (w/v) Difco-Bacto agar), 2% malt extract agar (2% (w/v) malt extract, 1.5% (w/v) Difco-Bacto agar), and PDA (4% (w/v)). Agars were chosen based on nutrient content and are listed from lowest nutrient content to highest nutrient level. Plates of each medium were sealed with parafilm (Parafilm M, American National Can) and assigned to 24-hour dark or 16-hour light growth chambers at a temperature of 21° C. Each growth chamber contained 12 VHO 4-foot fluorescent bulbs in combination with 4 40-watt incandescent bulbs approximately 2.5 feet above the plates. Colony diameter of *D. pini* on each plate was measured every five days for 35 days. On day 35, each plate was flooded with 10 ml deionized water and conidia were harvested by scraping with a rubber policeman. The water-fungus mixture was then filtered through 2 layers of autoclaved cheesecloth and an autoclaved porcelain Buchner funnel. Concentration of conidia was determined with a hemicytometer under a microscope.

This experiment was designed as a split plot with plates in a randomized complete block in each growth chamber and repeated three times. The first replication involved six plates per treatment per growth chamber and replications two and three had five plates per treatment per growth chamber. Plates were arranged in a single layer on a wire shelf in each growth chamber.

Data were analyzed with analysis of covariance and differences of least squares means (SAS, 1996). Conidia quantities were compared between PDA and all others after a log transformation. Statistical significance was determined for comparisons with $Pr>|t|=0.05$ using Tukey-Kramer separation due to difference in variance between replication 1 and replications 2 and 3.

Results

Radial growth of *D. pini* was most rapid on PDA (Fig. 1). Colony diameter among the other media tested was not significantly different. Potato dextrose agar also produced the most conidia after a 35-day period with a mean concentration of 42.25×10^{-2}C/ml (Table 1). Two percent malt extract had a significant amount of conidia when compared with other media and was different from all other media after comparison using the Tukey-Kramer test on data that were transformed to log of concentration of conidia to allow comparison (Table 1).

Light also had an effect on *D. pini.* Colony diameter of 2% malt extract agar and 1% malt extract agar was significantly larger in continuous darkness than in 16-hr light (Table 2). Colonies grown on potato dextrose agar were smaller in diameter when grown in 24-hour dark than in light. Conidia concentration was significantly higher on potato dextrose agar kept in 24-hour dark (Table 2). All other media showed no difference in conidia production between light and dark.

Discussion

Previous research on *D. pini* indicated that low-nutrient agars of 0.1% and 0.5% concentration of malt extract produced more conidia and less mycelium (Rack and Butin, 1973). Previous work also indicated that a high-nutrient agar (5.0% MEA) produced an abundance of mycelium but very few conidia at 1.0%, 2.0%, and 5.0% concentration of malt extract (Rack and Butin, 1973). At 10% concentration of malt extract conidia were hardly present but an abundance of mycelium was produced (Rack and Butin, 1973). However, in our study the low-nutrient agars (water agar, water agar with needle, and 1% malt extract agar) produced little mycelium and very few conidia and the high-nutrient agar (potato dextrose agar) produced the highest amount of both mycelium and conidia (Figure 1 and Table 1).

Light and dark had previously been reported to have no significant effect on colony size when using ordinary clear bulbs and fluorescent tubes, although earlier experiments showed a slight reduction in colony size in daylight (Ivory, 1967). In our study, light showed different effects on specific media. Ivory (1967) reported colony growth on only one medium (3% (w/v) malt extract agar) and had contradictory evidence on the effect of light, but he suggested that light had no effect on fungal growth. Our work showed that light and dark had significant effect on colony size on 1% and 2% malt extract agars and potato dextrose agar media (Table 2). Continuous darkness also produced significantly more conidia on potato dextrose agar than the 16-hr light treatment (Table 2). Although temperature of plates could have been higher under lights, we did not record temperature. However, we observed no condensation in plates that would indicate increased temperature.

Our study suggests that maximum mycelium growth occurs on PDA held under light while maximum conidia production occurs on PDA kept in the dark.

Literature Cited

Bradshaw, R.E., R.J. Ganley, W.T. Jones, and P.S. Dyer. 2000. High levels of dothistromin toxin produced by the forest pathogen *Dothistroma pini*. Mycological Research 104(3): 325-332.

Hulbary, R.L. 1941. A needle blight of Austrian pine. Illinois Natural History Survey Bulletin 21: 230-236.

Ivory, M.H. 1967. Spore germination and growth in culture of *Dothistroma pini* var. *keniensis*. Transactions of the British Mycological Society 50(4): 563-572.

Rack, K. and H. Butin. 1973. A quick method for the production of *Dothistroma pini* spores in culture. European Journal of Forest Pathology 3: 201-209.

SAS Institute, Inc. 1996. SAS User's Guide.

Table 1. Analysis of variance of number of *D. pini* conidia for cultures on different media after 35 days with combined light treatments.

Media	Concentration of Conidia ($Cx10^{-2}ml$)	DF	t-value	Pr>|t|
Water agar	0. 05^{z}	81	0.03	0.98
Water agar with needle	0. 2	81	0.10	0.92
2% Malt extract agar	4.0	81	2.05	0.04
1% Malt extract agar	0. 05	81	0.03	0.98
Potato dextrose agar	42.25	81	21.63	<.0001

^{z}N=22

Table 2. Comparison of the effect of 24-hr darkness versus 16 hr-light on colony diameter and number of conidia for each media. Means were separated with one-way analysis of variance with statistical significance at $P<0.05$.

	Colony diameter at day 35 (mm)			Number of conidia ($Cx10^{-2}ml$)		
Media	Light	Dark	Pr>F	Light	Dark	Pr>F
Water agar	15.5a[z,y]	16.7a	0.6	0.1a[x,y]	0.0a	0.3
Water agar with needle	17.5a	16.6a	0.7	0.3a	0.1a	0.3
2% Malt extract agar	12.8a	19.3b	0.0002	3.5a	4.5a	0.5
1% Malt extract agar	11.6a	22.4b	0.0002	0.0a	0.1a	0.3
Potato dextrose agar	43.0a	37.9b	0.02	31.8a	52.7b	0.03

[z]Means separated between light and dark for colony diameter. Means with the same letter are not significantly different at $p<0.05$.

[y]N=16 per media per treatment.

[x]Means separated between light and dark for number of conidia. Means with the same letter are not significantly different at $p<0.05$.

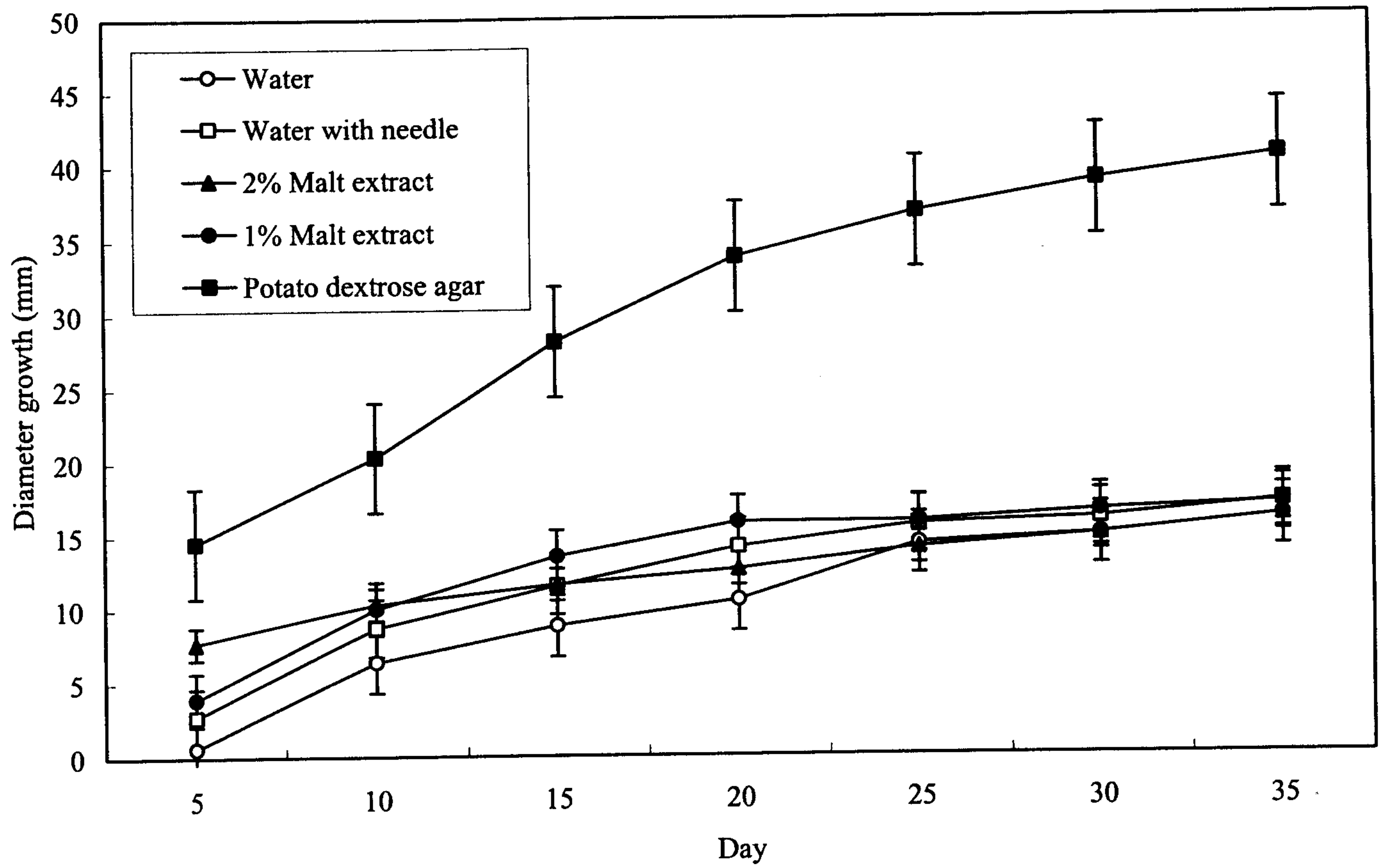

Figure 1. Mean colony diameter on 5 different media over 35 days.

Chapter 3. Susceptibility of Bosnian and Korean pines to Dothistroma needle blight

A paper to be submitted to *Journal of Environmental Horticulture*

Carol D. La Faver, Janette R. Thompson, Jeffery K. Iles, and Mark L. Gleason

Significance to the Nursery Industry

Moisture extremes, insect and nematode pests, and fungal pathogens have combined to limit the range of conifer species commonly used in amenity plantings and windbreaks in Iowa and much of the Midwestern United States. This reality has prompted nursery and landscape professionals to consider adding other little-known or under-used conifers to their plant palette. Responding to this need, we studied the susceptibility of Bosnian pine (*Pinus leucodermis* Ant.) and Korean pine (*Pinus koraiensis* Sie. & Zucc.) to Dothistroma needle blight (*Dothistroma pini* Hulbary). In this inoculation study, both Bosnian and Korean pine seedlings became infected by *Dothistroma pini*. The pattern and severity of infection of these species were similar to those of Austrian pine (*Pinus nigra* Arn.). Our study suggests that field trials of Bosnian pine and Korean pine should be conducted before widespread planting in the landscape, and that individual specimens should be screened for pathogen susceptibility.

Abstract

The range of conifer species appropriate for Midwest landscapes are extremely limited due to climatic, pest, and pathogen pressures. For this study, we identified two under-used conifers with potential for increased use: Bosnian pine (*Pinus leucodermis* Ant.) and Korean pine (*Pinus koraiensis* Sie. & Zucc.). Our objectives were to determine the susceptibility of Bosnian pine and Korean pine to *Dothistroma pini* Hulbary, and to compare their performance to Austrian pine (*Pinus nigra* Arn.), which is known to be

susceptible. Seedlings were inoculated with a conidial suspension, covered with an opaque bag and placed in a growth chamber with a 16-hour photoperiod and day/night temperatures of 24°/17° C for 24 hours. Trees were then placed in a greenhouse and incidence and severity of infection were observed for 90 days. Growth and physiological parameters, including photosynthetic rates and percent starch in roots, were measured. All species became infected when inoculated with *D. pini*, and there were no significant differences among species in incidence or severity of infection. No differences were detected in physiological responses of inoculated versus control seedlings for each species. Our findings suggest that Bosnian pine and Korean pine should be more rigorously screened before they are strongly recommended for landscape plantings in the upper Midwest.

Introduction

Conifer choices for the central United States are severely limited due to climatic, pest, and pathogen pressures. Several pines previously planted throughout the Midwest (for example Scot's pine and Austrian pine) are no longer recommended for use in the region because of disease problems, which further limits the choice of evergreen plant material (Gleason et al., 2000). The lack of suitable conifers is especially problematic in Iowa, which has only three native conifer species. This study was initiated to screen large landscape-size conifers that are not common in the upper Midwest to determine their potential for more widespread use.

Dothistroma pini Hulbary was first identified in 1941 as the cause of Austrian pine needle blight (Hulbary, 1941). This foliar blight affects over 30 species of conifers in the genera *Pinus*, *Larix*, *Picea*, and *Psuedotsuga* in landscape, park, forest, plantation, and windbreak settings (Kershaw et al., 1982). *D. pini* is now known to occur in Australia, Europe, Asia, North America, South America, and Africa (Murray and Batko, 1962; Peterson, 1969; Edwards and Walker, 1978; Kershaw et al., 1982). In the United States, 22 of the 50 states have reported infection of *Pinus* by *D. pini* (Peterson, 1967a). Thus, it is a major concern in conifer plantings. Two applications of copper based fungicides are recommended for control of the fungus (Peterson, 1965; Peterson, 1967b; Peterson, 1969; Peterson, 1981; Kershaw et al., 1982).

The two experimental taxa in this study, Bosnian pine and Korean pine, have not appeared on lists of species susceptible to Dothistroma needle blight. They were chosen for investigation based on observations of healthy, mature specimens in various arboreta and landscape settings in the Midwest (Thompson and La Faver, 2002).

Bosnian pine is very similar to Austrian pine in morphology and growth (Krussman, 1985; Rushforth, 1987). Native to areas of Yugoslavia, Albania, Bulgaria, Greece, and Italy, Bosnian pine is a 2-needled pine that can grow to a 30-m height, maintaining a dense pyramidal shape (Krussman, 1985). Although Austrian pine and Bosnian pine are quite similar, trees of Bosnian pines tend to have darker green and less resinous needles than Austrian pines (Krussman, 1985).

Korean pine, is native to areas of Korea and Japan, and also grows to heights of 20 to 30 m (Krussman, 1985; Rushforth, 1987; Dirr, 1990). Korean pine is a 5-needled pine that has a loose, pyramidal shape similar to that of Eastern white pine (*Pinus strobus*). The needles of Korean pines have a grey-green color that adds interest in the landscape (Dirr, 1990). Korean pine is recommended for planting as far north as USDA hardiness zone 3 and is considered one of the hardiest conifers available (Krussman, 1985; Dirr, 1990).

One objective of this study was to compare susceptibility of Bosnian pine and Korean pine to *Dothistroma pini* with that of Austrian pine, which is known to be susceptible. A second objective was to measure physiological response to the pathogen, specifically photosynthetic rate and starch storage in root tissue. Our hypothesis was that Bosnian pine and Korean pine would be less susceptible to infection than Austrian pine. We also hypothesized that infected seedlings would have lower photosynthetic rates and diminished starch storage than healthy seedlings.

Materials and Methods

Four-liter (#1) container-grown seedlings of Bosnian pine and Austrian pine were purchased in spring 2001 from Alpha Nursery (Silverton, OR) and Bailey Nursery (St. Paul, MN) respectively. Austrian pine was included in the study because it is known to be susceptible to *D. pini*. Also in spring 2001, bareroot Korean pine (3-0) from Heritage Seedlings, Inc. (Salem, OR) were potted in 4-L containers (#1) using Fafard® mix #51 (65% bark, 35% peat and perlite). Trees were maintained in a greenhouse under 16 hour days and 25°C until the experiment began. In late 2001, 5 trees per species were sprayed with a 15 ml conidial suspension of *D. pini* Hulbary using an atomizer as described by Gadgil (1974). The suspension concentration was between $4x10^6$ (conidia/ml) and $7x10^6$ conidia/ml (Parker, 1972; Gadgil, 1974; Gadgil, 1977). Five trees per species were treated with 15ml of deionized water to serve as a control. Each tree was covered with an opaque plastic bag with the interior sprayed with 5 ml deionized water. The bags were supported by wire to prevent direct contact with the foliage (Parker, 1972). Each tree was then randomly assigned to one of 2 growth chambers, and held for 24 hours at day/night temperatures of 24°/17° C and a 16-hour photoperiod (Peterson, 1967a; Gadgil, 1974, and Gibson, 1974). After the bags were removed, trees were placed in the greenhouse at 25° C, and monitored twice weekly for 90 days for symptom appearance.

Observations of each tree included time of appearance of first banding (Gadgil and Holden, 1976), percent needles showing banding, and a rating of severity of infection based on number of lesions per needle rated on a rising 3-point scale as described by Peterson (1973, 1984) with 0 lesions=0 rating, 1 lesion per needle=1 rating, 2-5 lesions per needle=2, and 6 or more lesions per needle=3 rating. At approximately 45 and 90 days, photosynthesis measurements were taken with a LI-COR 6400™ (Li-Cor Inc., Lincoln, NE) fitted with a conifer chamber. Time of day, light, and temperature each time photosynthesis measurements were taken were similar. Following photosynthesis measurements on day 90, plants were harvested, separated into needles, stems, and roots, and oven-dried at 67° C for 144 hours. Roots were processed through a Wiley Mill grinder to pass a 0.5-mm mesh sieve and were analyzed for percent starch content by Servi-Tech Laboratories using the amyloglucosidase enzymatic method (Omi and Rose, 1991) and a YSI 2700 Biochemistry analyzer (Hastings, Nebraska).

There were three replications with five experimental units for each treatment for Bosnian pine and Austrian pine and one replication with five experimental units for each treatment for Korean pine. Korean pines were used for only one replication due to difficulty in maintaining seedling health in container culture. Each experiment was designed as a randomized complete block and analyzed using one-way analysis of variance in JMP statistical software (SAS Institute, Cary, NC). Statistical significance was determined for comparisons with $P<0.05$.

Results

Our hypothesis regarding species susceptibility was not supported by the results of this inoculation study. There were relatively few differences detected within a species for means of height or diameter for inoculated versus control seedlings (Table 1). Inoculated seedlings of Bosnian pine were significantly taller at 90 days than the Bosnian pine control seedlings (Table 1).

No significant differences were found between species for first sampling day symptoms were observed; however, Korean pine showed symptoms after an average of 6.8 days, Bosnian pine at 12.6 days, and Austrian pine at 15.7 days (Table 2). Percent infected needles for inoculated seedlings after 90 days ranged from 6.4% (Austrian pine) to 24.0% (Korean pine), although again no significant differences were detected between species (Table 2). Infection severity ratings at the end of 90 days were similar with a range from 0.9 for Austrian pine to 1.5 for Bosnian pine (Table 2), although no significant differences were detected.

The pattern of infection for all three species was similar (Figures 1 and 2). Although symptoms were noticed first on Korean pine, the proportion of needles affected and number of lesions per needle were less than those observed for Bosnian pine up to sample date 8 (approximately day 28). The percent of needles affected increased steadily for Bosnian pine after sample date 5, for Korean pine after sample date 9, and for Austrian pine after sample date 20. Progression of infection severity (number of lesions per needle) followed a more episodic pattern, with increases in severity occurring between sample dates 4-8, 10-14, and 22-23 for Bosnian pine and Korean pine and on the latter date for Austrian pine.

No differences between inoculated and control seedlings within each species were detected for dry weights of different plant parts, photosynthetic rates or root starch storage at the end of 90 days (Table 3).

Discussion

This study demonstrates that Bosnian pine and Korean pine are susceptible to *Dothistroma pini*, which to our knowledge has not been previously reported. Peterson (1967) suggested that 2-, 3-, and 5-needled pines, particularly exotics, in the Midwest United States could be susceptible to *Dothistroma pini*. Austrian pine is well known as a frequent host for D. pini. Bosnian pine, which is morphologically similar to Austrian pine, demonstrated similar susceptibility to *D. pini* (Table 2). It has also been reported that Eastern white pine can be susceptible although it is much less frequently a problem for landscape plants of this species than for Austrian pine (Peterson, 1967; Kershaw et al; 1982). Korean pine is more closely related to Eastern white pine, but demonstrated infection levels comparable to Austrian pine and Bosnian pine under optimum conditions for fungal infection (Table 2). However, this infection percentage and severity may be related to the difficulty of maintaining Korean pine in container culture.

Differences in seedling size between species were primarily attributable to differences in stock type and seedling age. Size and growth rate data did not indicate a consistent or negative effect of inoculation and subsequent infection on seedling performance in a 90-day evaluation period.

The patterns of infection for the three species were very similar (Figures 1 and 2). Both infection percent and infection severity increased more rapidly for Bosnian pine and Korean pine compared to Austrian pine. This also suggests that the experimental taxa would have similar susceptibility to *D. pini* as Austrian pine.

Although we did not detect differences due to infection in seedling growth and physiological parameters, trends in the data suggest that a longer study may have detected differences for these parameters. The infection data strongly point toward a progression in the disease that would lead to decreased photosynthetic capacity and ultimately decreased growth rates.

Similar susceptibility and pattern of infection by *D. pini* indicate that the experimental taxa may require additional screening before they can be recommended for widespread planting in the Midwest. Similar screening of additional species may aid in identification of *Pinus* species that are better suited for use the upper Midwest.

Literature Cited

Dirr, M.A. 1990. Manual of Woody Landscape Plants: Their Identification, Ornamental Characteristics, Culture, Propagation, and Uses. 4th ed. Stipes, Champaign, IL.

Edwards, D.W. and J. Walker. 1978. Dothistroma needle blight in Australia. Australian Forest Research 8: 125-137.

Gadgil, P.D. 1974. Effect of temperature and leaf wetness period on infection of *Pinus radiata* by *Dothistroma pini*. New Zealand Journal of Forestry Science 4(3): 495-501.

Gadgil, P.D. 1977. Duration of leaf wetness periods and infection of *Pinus radiata* by *Dothistroma pini*. New Zealand Journal of Forest Science 7(1): 83-90.

Gadgil, P.D., and G. Holden. 1976. Effect of light intensity on infection of *Pinus radiata* by *Dothistroma pini*. New Zealand Journal of Forest Science 6(1): 67-71.

Gibson, I.A.S. 1974. Impact and control of dothistroma blight of pines. European Journal of Forest Pathology 4: 89-100.

Gleason, M.L., M. Linit, N. Zriba, P. Donald, N. Tisserat, and L. Giesler. 2000. Pine wilt: A fatal disease of exotic pines in the Midwest. Iowa State University. University Extension SUL-9, Ames, IA.

Hulbary, R.L. 1941. A needle blight of Austrian pine. Illinois Natural History Survey Bulletin 21(7): 231-236.

Kershaw, D.J., P.D. Gadgil, G. J Leggat, J.W. Ray, and J. B. van der Pas. 1982. Assessment and control of Dothistroma needle blight revised edition. FRI Bulletin No. 18.

Krussman, G. 1985. Manual of Cultivated Conifers. Timber Press, Portland, OR.

Murray, J.S. and S. Batko. 1962. *Dothistroma pini* Hulbary: A new disease on pine in Britain. Forestry 35: 57-65.

Omi, S.K. and R. Rose. 1991. Amyloglucosidase and α–amylase enzymes for determining starch concentration in Ponderosa pine root tissue. Forest Science 37(5): 1479-1483.

Parker, A.K. 1972. Artificial inoculation of *Pinus radiata* with *Scirrhia* (*Dothistroma*) *pini*: Effect of relative humidity and temperature on incubation. Phytopathology 62: 1160-1164.

Peterson, G.W. 1965. Dothistroma needle blight of Austrian pine: Infection and control. Plant Disease Reporter 49(2): 124-126.

Peterson, G.W. 1967a. Dothistroma needle blight of pines in North America. Exposes XIV IUFRO Kongress: 269-278.

Peterson, G.W. 1967b. Dothistroma needle blight of Austrian and Ponderosa pines: Epidemiology and control. Phytopathology 57: 437-441.

Peterson, G.W. 1969. *Dothistroma pini* needle blight on *Pinus radiata*. FAO Plant Protection Bulletin 17: 43.

Peterson, G.W. 1973. Infection of Austrian and Ponderosa pines by *Dothistroma pini* in eastern Nebraska. Phytopathology 63: 1060-1063.

Peterson, G.W. 1981. Control of diplodia and dothistroma blights of pines in the urban environment. Journal of Arboriculture 7(1): 1-5.

Peterson, G.W. 1984. Resistance to *Dothistroma pini* within geographic seed sources of *Pinus ponderosa*. Phytopathology 74(8): 956-960.

Rushforth, K.D. 1987. Conifers. Bath Press, Bromley, Kent, England.

Thompson, J.R and C.D. La Faver. 2002. The Quest for Conifers. The Ames Forester, Iowa State University, Ames, IA.

Table 1. Means for initial, final, and incremental height and diameter compared between control and inoculated seedlings within species.

Species	Initial height (cm)	Final height (cm)	Height growth (cm)	Initial diameter (mm)	Final diameter (mm)	Diameter growth (mm)	N^z
P. leucodermis							
Inoculated	27.7a	30.1a	2.5a	12.7a	14.9a	2.1a	15
Control	26.5a	27.2b	0.9a	12.9a	14.9a	2.3a	15
		(n=14)	(n=14)		(n=14)	(n=14)	
d.f.	1	1	1	1	1	1	
F	1.62	4.78	2.63	0.13	0.0	0.20	
P	0.21	0.04	0.12	0.72	0.99	0.66	
P. koraiensis							
Inoculated	29.7a	29.6a	-0.1a	7.3a	8.1a	0.8a	5

Table 1. continued

Control	31.3a	31.4a	0.1a	6.8a	7.7a	0.9a	5
d.f.	1	1	1	1	1	1	
F	0.23	0.38	0.00	0.27	0.15	0.03	
P	0.65	0.56	0.95	0.62	0.71	0.87	
P. nigra							
Inoculated	31.1a	31.4a	0.4a	12.9a	15.9a	3.0a	15
Control	30.9a	33.3a	2.4a	13.2a		2.6a	15
					15.8		
d.f.	1	1	1	1	1	1	
F	0.02	0.99	1.41	0.90	0.04	0.68	
P	0.89	0.33	0.25	0.36	0.84	0.42	

[z]Deviations in sample numbers are noted parenthetically for some means.

Table 2. Appearance of symptoms, infection percent and infection severity for three species of *Pinus* inoculated with *Dothistroma pini*.

Species	Earliest detection of symptoms[z]	Infection (%)[y]	Infection severity[x]	N
P. leucodermis	12.6a[w]	14.5a	1.5a	13
P. koraiensis	6.8a	24.0a	1.4a	4
P. nigra	15.7a	6.4a	0.9a	15
d.f.	2	2	2	
F	2.5	2.6	2.3	
P	0.10	0.08	0.11	

[z]Mean number of days from inoculation to detection of first symptoms.

[y]Percent of total needles showing signs/symptoms at 90 days.

[x]Infection severity based on average number of lesions per needle at 90 days.

[w]Means within a column with the same letter are not different at $p<0.05$.

Table 3. Dry weight, photosynthesis rate, and root starch content by species for control and inoculated seedlings of three *Pinus* species.

	Dry Weight (g)			Photosynthesis ($\mu MCO_2m^{-2}s^{-1}$)		Starch (%)	N[z]
Species Treatment	Roots	Stems	Needles	Day 45	Day 90		
P. leucodermis							
Inoculated	77.8a[y]	30.3a	71.1a	14.7a (n=10)	10.4a	2.9a	15
Control	82.4a	29.6a	77.7a	15.8a (n=10)	10.1a	2.9a	15
d.f.	1	1	1	1	1	1	
F	0.23	0.1	1.04	0.18	0.02	0.008	
P	0.64	0.75	0.31	0.68	0.88	0.93	
P. koraiensis							
Inoculated	6.4a	5.17a	9.9a	6.3a	5.0a	4.6a	5
Control	5.9a	5.5a	9.8a	3.7a	6.1a	4.6a	5
d.f.	1	1	1	1	1	1	
F	0.07	0.06	0.007	0.92	0.09	0.0005	
P	0.79	0.81	0.93	0.37	0.78	0.99	

Table 3. continued.

P. nigra							
Inoculated	61.1a	28.8a	53.5a	13.8a (n=10)	12.6a	2.5a	15
Control	71.1a	28.2a	50.9a	14.7a (n=10)	14.4a	2.4a (n=14)	15
d.f.	1	1	1	1	1	1	
F	1.04	0.11	0.5	0.15	0.70	0.007	
P	0.32	0.75	0.49	0.70	0.41	0.94	

[z]Deviations in sample numbers are noted parenthetically for some means.

[y]Means within a column with the same letter are not significantly different at $p<0.05$.

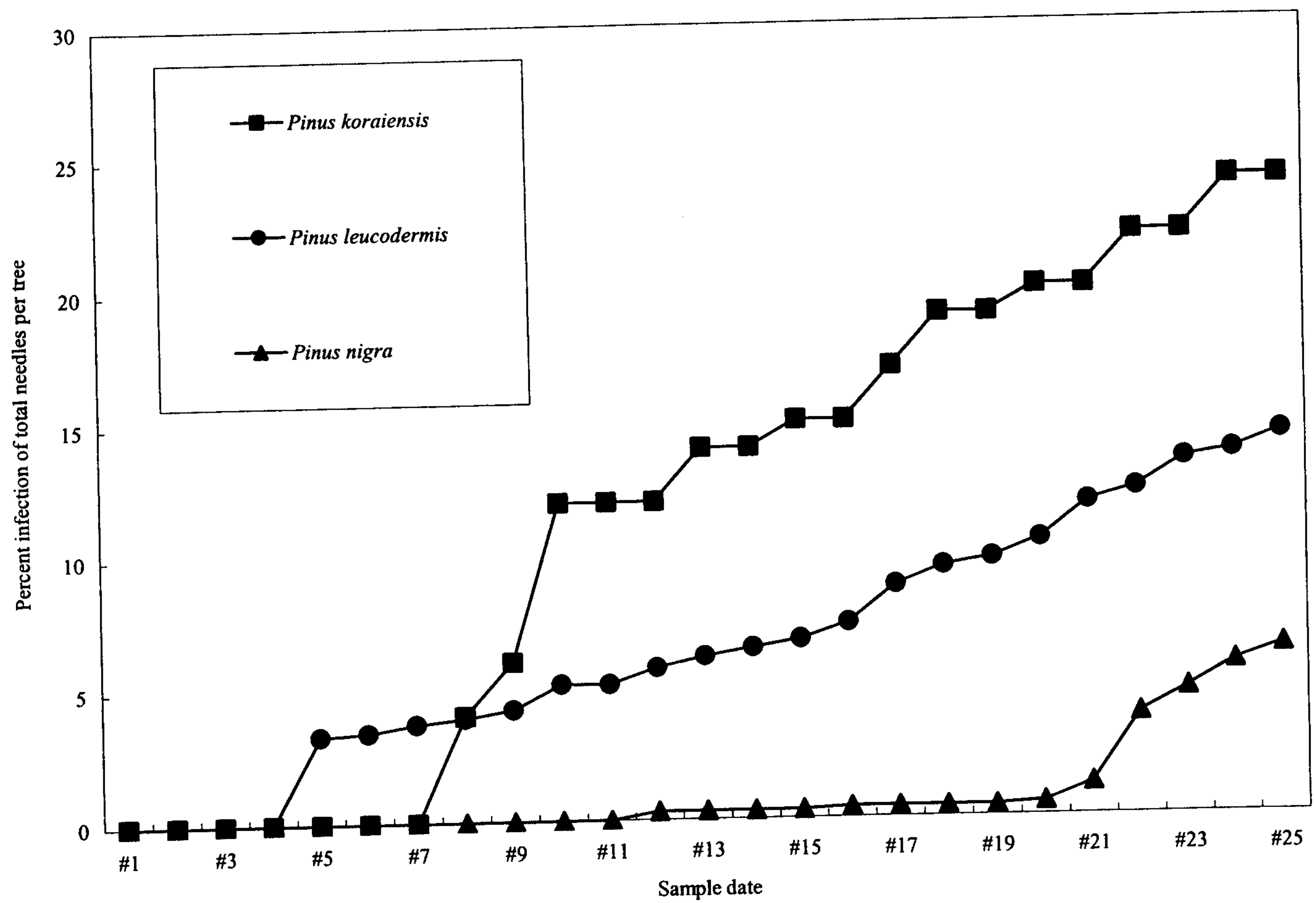

Figure 1. Mean percentage of infection (proportion of needles showing symptoms) of *Pinus* species by *D. pini*. Infection percent was determined visually twice weekly for 90 days.

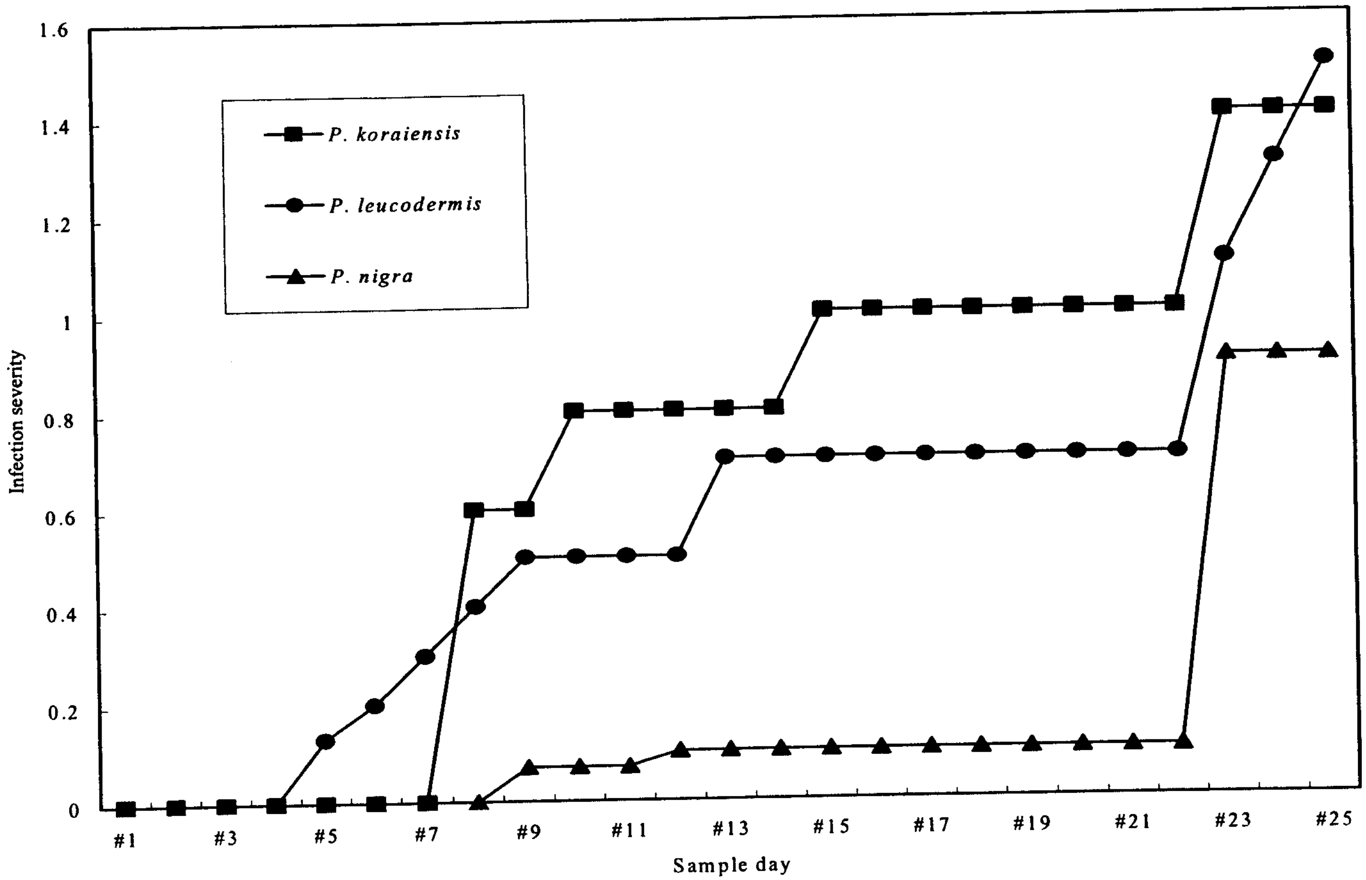

Figure 2. *D. pini* infection severity for three *Pinus* species. Infection severity was rated on a rising 1-3 scale based on average number of lesions per infected needle (Peterson, 1973, 1984) and data presented are means for each species.

Chapter 4. Survival and growth of Nikko fir in Iowa

A paper to be submitted to *Landscape Plant News*.

Carol D. La Faver and Janette R. Thompson

Abstract

Conifers in Iowa are subjected to extreme climatic conditions and pest and pathogen pressures limiting the number of conifer species that successfully grow in the landscape. The objective of this study was to begin to assess the performance of Nikko fir (*Abies homolepis*), a plant that has not been commonly used in the upper Midwest. Nikko fir and Norway spruce (*Picea abies*) were outplanted at a site in Ames, Iowa. Survival and canopy and stem growth data were collected over an 18-month period (two growing seasons). Although there was a "row effect" on survival based on placement of seedlings of the two species at the field site, statistical analysis indicated there were no differences in survival or growth between species. With appropriate post-transplant care, Nikko fir may be suitable for use in the Iowa landscape.

Introduction

The number of conifer species successfully used in central Iowa has been limited due to moisture and temperature extremes, insect and nematode pests, and fungal pathogens (Gleason et al., 2000). Unfortunately, only three species of conifer trees are native to Iowa: eastern white pine (*Pinus strobus*), eastern red cedar (*Juniperus virginiana*), and balsam fir (*Abies balsamea*) (van der Linden and Farrar, 1984).

Such limited choices among conifers are disappointing for nursery and landscape professionals and homeowners looking for suitable landscape plant material. We began our search for "new" conifers with potential for more widespread use in the upper Midwest by visiting 7 arboreta/plant collections in the Midwest. Data collected during these visits included height, width of tree canopy at the base, DBH, longevity of needles, needle color, tree shape, presence and characteristics of cones, and accession dates (when available). Data from more than 56 species were collected from the genera *Abies, Picea*, and *Pinus* (Thompson and La Faver, 2002). After assessing our initial data, we chose to further investigate the performance of *Abies homolepis* (Nikko fir).

Abies homolepis Siebold and Zuccarini, is an ornamental fir native to Japan (Rushforth, 1987). The Nikko fir is known for its highly aesthetic appeal in the landscape as well as its ability to thrive under extreme conditions (Dirr, 1990; Rushforth, 1987). Nikko fir is known for the contrasting dark green older needles and light green new foliage that is particularly striking in the landscape (La Faver, 2002).

Picea abies L. Karst. (Norway spruce) is a known landscape tree that consistently thrives in Iowa landscapes. Norway spruce is hardy in USDA zones 2-7 and thrives in a wide variety of sites (Krussman, 1985; Dirr, 1990).

The objectives of this study were to assess survival and canopy and stem growth of *Abies homolepis* in central Iowa and to evaluate the suitability of this species for more general use in the Midwestern landscape. In this study, we hypothesized that growth and survival of *A. homolepis* would be similar to *P. abies*.

Materials and Methods

Plug seedlings of *Abies homolepis* (P2) and bareroot *Picea abies* (3-0) seedlings were obtained from Western Maine Nursery (Fryeburg, MI) and Lawyers Nursery (Plains, MT), respectively, in fall 1999. Seedlings were potted with 1 seedling per 4.5-L containers using Fafard #51 pine bark mix (Fafard, Inc., Agawam, MA). Trees were arranged pot-to-pot, surrounded with straw bales, and covered with white plastic for overwintering at the Iowa State University Horticulture Research Station. In June 2000, all trees were planted by hand at a field research site in Ames, Iowa. A 3% solution of Round-Up® (glyphosate, Monsanto, St. Louis, MO) was sprayed with a backpack sprayer on grass species to decrease competition from existing vegetation. Survival, total height, new terminal growth, base lateral growth, diameter, and number of secondary branches were measured in Fall 2000, Spring 2001, and Fall 2001. Damage due to deer browse also was noted. Diameter was measured with digital calipers. Samples of soil from the surface to 15 cm deep were collected every 1.22 m along a transect across the plot and analyzed to determine particle size using a modified pipette method (Gee and Bauder, 1986) at the Iowa State University Soil Survey lab. Precipitation data were obtained from Iowa State University Agronomy Department (Dennis Todey, personal communication, 2002).

Trees were planted in rows without randomization of species with rows 1-3 and 9-11 containing Nikko fir and rows 4-8 planted in Norway spruce. Canopy and stem measurements were pooled to obtain mean values for surviving trees. A logistic regression procedure was used to compare survival of species. Means were determined and growth data were analyzed with a one-way analysis of variance in JMP statistical software (SAS Institute, Cary, NC).

Results

In Fall 2000, 3-month survival rates of Nikko fir and Norway spruce were 67% and 92%, respectively, since planting. In Fall 2001, 18-month survival rates were 45% for Nikko fir and 89% for Norway spruce. The row in which seedlings were placed affected survival significantly. For Nikko fir in rows 1-3 for fall 2000, spring 2001, and fall 2001 survival was 92.2%, 38.8%, and 26.6% respectively. Survival for rows 9-11 for the same time periods were 100%, 91.2%, and 88.2% respectively.

No differences were found in growth between species in canopy and stem measurements (Table 1). In Spring 2001 total height of Norway spruce decreased, probably due to deer browse, while total height of Nikko fir increased.

Analysis of soil particle size distribution indicated very little difference across the site. Precipitation data for two growing seasons was below the 10-year average (Fig. 2).

Discussion

Survival was apparently influenced by row of placement, especially for Nikko fir (Fig. 1). Unfortunately we were unable to identify a single factor to explain the row effect, however the combination of several factors (differences in soil moisture holding capacity, incident light levels, below average precipitation, and above average temperatures) may have contributed to mortality of the Nikko fir in the first three rows.

We are not aware of previous field tests for the Nikko fir in the continental United States other than occasional specimens in arboreta. *Abies homolepis* were among the species monitored by Danby and Mason (1998) in the Brechfa forest experimental plots located in South Wales, Great Britain. Although the Brechfa trees were planted for analysis of potential for forestry production, their condition was rated as fair after 34 years in a relatively mild climate and they had reached an average height of 16.9 m and diameter of 20.9 cm (Danby and Mason, 1998). Our preliminary results indicate that Nikko fir had similar growth to Norway spruce, so it may be a suitable plant for more general use in the Iowa landscape. Due to site sensitivity of fir species, further screening and testing of this species over a longer time period is suggested before recommending it for more widespread distribution by the nursery and landscape industry.

Literature Cited

Danby, N. and B. Mason. 1998. The Brechfa forest plots: Results after 40 years. Quarterly Journal of Forestry 92: 141-152.

Dirr, M.A. 1990. Manual of Woody Landscape Plants. Their Identification, Ornamental Characteristics, Culture, Propagation, and Uses. 4th ed. Stipes, Champaign, Ill.

Gee, G.W. and J.W. Bauder. 1986. Particle-size analysis, pp. 383-411. In Klute, A. (ed.). Methods of Soil Analysis Part 1. 2nd ed. Book series #5, ASA and SSSA, Madison, WI.

Gleason, M.L., M. Linit, N. Zriba, P. Donald, N. Tisserat, and L. Giesler. 2000. Pine wilt: A fatal disease of exotic pines in the Midwest. Iowa State University. University Extension SUL-9, Ames, IA.

Krussman, G. 1985. Manual of Cultivated Conifers. Timber Press, Portland, OR.

La Faver, C. D. 2002. Overlooked conifers for the Midwest. Conifer Quarterly 19: 15-17.

Rushforth, K. D. 1987. Conifers. Bath Press, Bromley, Kent.

Thompson, J.R. and C.D. La Faver. 2002. The Quest for Conifers. The Ames Forester, Iowa State University, Ames, IA.

van der Linden, P., and D. Farrar. 1984. Forest and Shade Trees of Iowa. Iowa State University Press: Ames, IA.

Table 1. Canopy and stem measurements on *Abies homolepis* (P2) and *Picea abies* (3-0) planted in 2000 in central Iowa. All measurements were taken over an 18-month period on surviving trees. Means of surviving trees in each column were based on 99 *A. homolepis* and 122 *P. abies* in the fall 2000 and 67 *A. homolepis* and 119 *P. abies* in fall 2001.

	Total height (cm)		New terminal growth (cm)		Lateral length (cm)		Diameter (mm)		
	Fall 2000	Fall 2001	Fall 2000	Fall 2001	Fall 2000	Fall 2001	Fall 2000	Spring 2001	Fall 2001
Species									
Abies homolepis	18.1	26.2	8.5	7.9	9.9	10.5	4.8	5.6	6.9
Picea abies	42.4	36.9	9.9	10.6	8.9	21.4	6.8	7.1	8.6

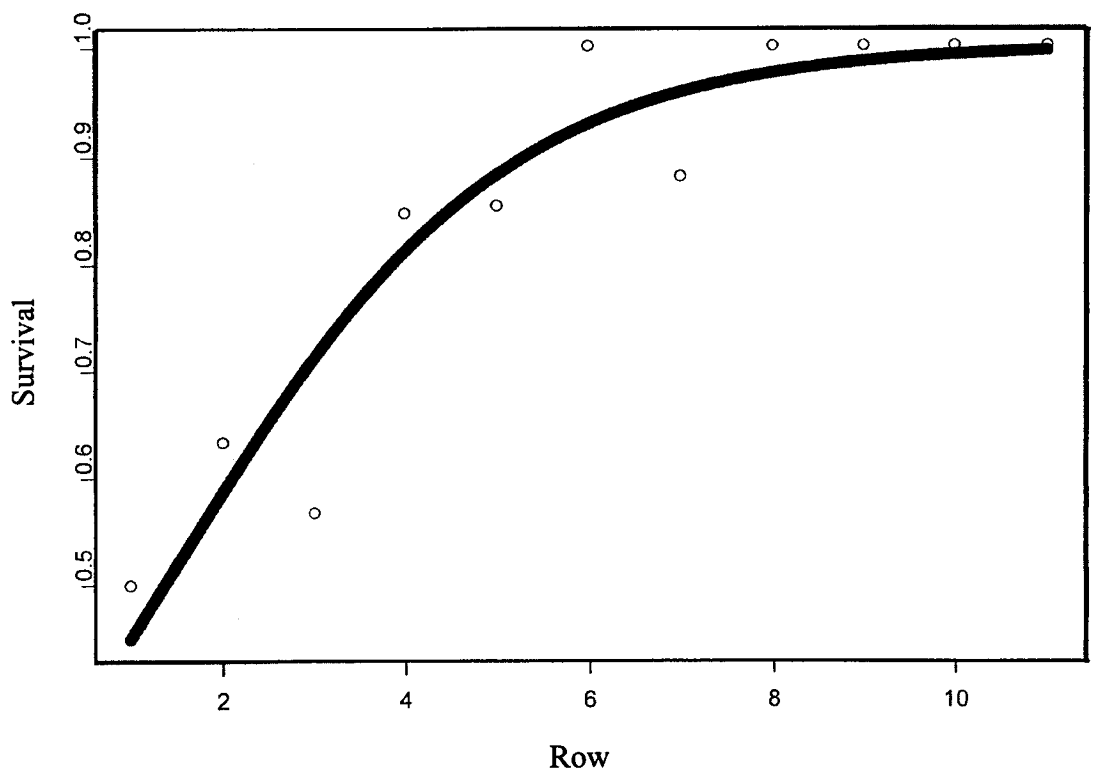

Figure 1. Survival of trees across rows. Row (location in the field) had a significant effect on seedling survival using a logistic regression procedure ($p<0.0001$).

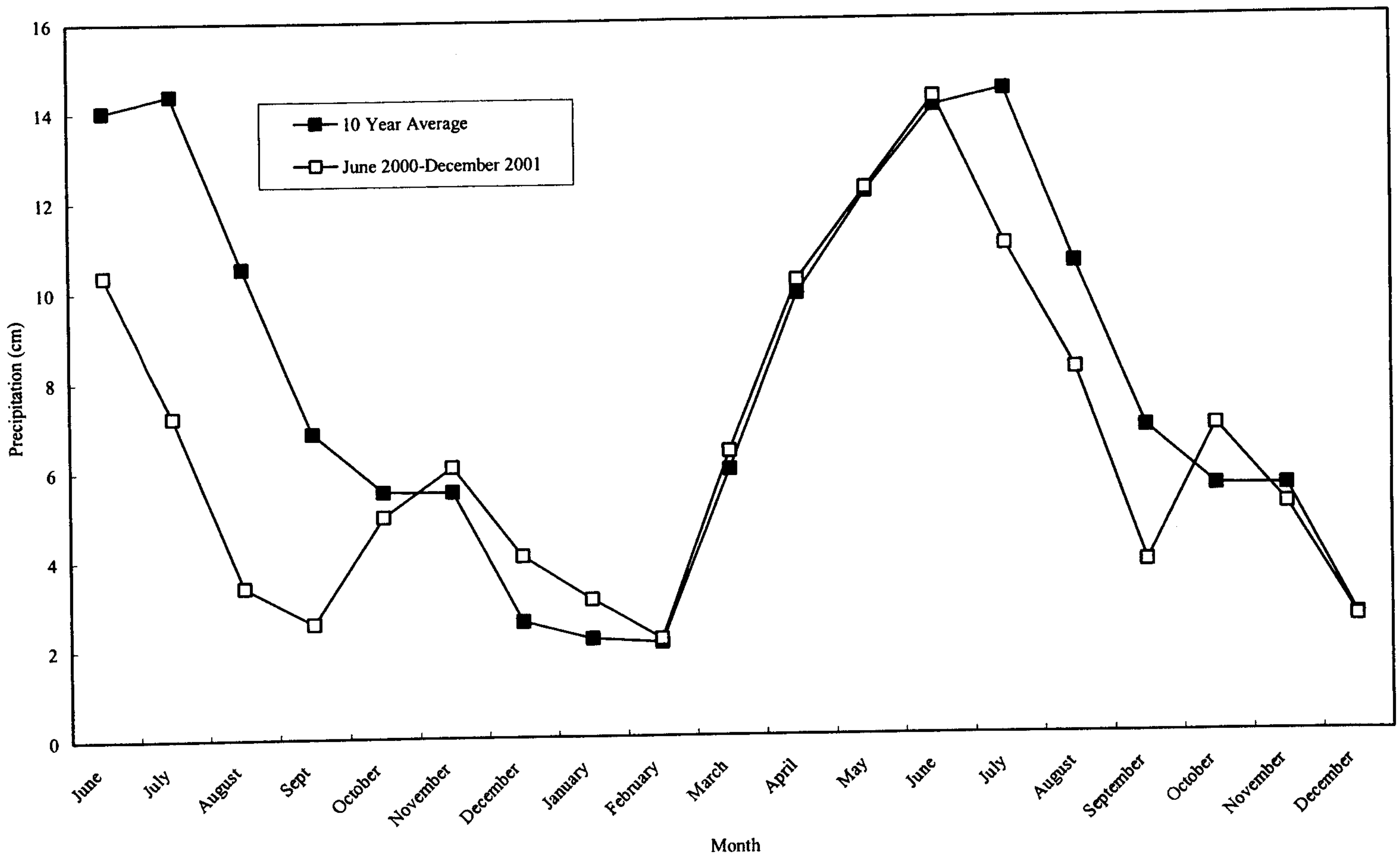

Figure 2. Mean monthly precipitation in Ames, Iowa over an 18-month period. 10-year monthly average between 1991-2000 are reported as well as the monthly average precipitation during this study (June 2000-December 2001). All monthly precipitation is reported in cm.

Chapter 5. General Conclusions

General Discussion

The limited number of conifer species for the landscape continues to constrain nursery and landscape professionals in Iowa and much of the Upper Midwest. During the course of this project we attempted to broaden the palette of conifers available for use in landscapes and windbreaks. The combination of these three projects is an initial step assisting the industry and providing recommendations for testing more conifers for Iowa.

When we began culturing *Dothistroma pini*, the rate of growth was relatively unknown. We found that contrary to previous research, nutrient-rich media (PDA) was most effective in growing mycelium and producing conidia (see Chapter 2). Although media that was used successfully in prior research was also utilized in this study, cultures grown on PDA had consistently and significantly larger diameter and more conidia. We also confirmed previous work that light does limit colony size and conidia formation on specific media.

Susceptibility of *Pinus leucodermis* and *Pinus koraiensis* to *D. pini* was confirmed in this work (Chapter 3). Not only were the experimental taxa susceptible, but the infection pattern of these species followed that of *P. nigra,* which is known to be highly susceptible. Although no physiological differences occurred between control and inoculated plants for any species tested, trends could be detected, especially in photosynthetic rates. Trends in photosynthetic rate were emerging according to the pattern we expected, with a decrease in inoculated plants between days 45 and 90. If the study was continued over a longer period of time, we expect that a significant decrease would have been detected in inoculated plants, due to necrosis of tissue that follows the banding symptoms we observed. Root tissue starch levels may have also began to differentiate after a longer period of time; however, since no differences were found in photosynthesis, it was not surprising to find no differences for starch levels in this study. Although experimental taxa were susceptible and may not be suitable for widespread planting, these species may still be suitable for specimen plantings in the landscape.

Abies homolepis also may be suitable for the Midwest landscape. The survival and growth performance of *A. homolepis* in comparison to *Picea abies* showed that it has potential for planting (Chapter 4). Further analysis and screening of this species is recommended before widespread distribution by the nursery and landscape industry.

Recommendations for Future Research

Many interesting questions arose as a result of this research. In his book *Conifers*, Rushforth (1987) mentions that *A. homolepis* is particularly tolerant of urban pollution; however, no literature has been found to support this statement. Future research on this species should include studies looking at physiological response to salt, air, and/or soil pollutants. If truly tolerant of pollutants, this tree could have great potential for highly urbanized areas with limited species for planting.

Pinus leucodermis has been long been compared to *Pinus nigra*. Now that *P. leucodermis* is confirmed to be susceptible to *D. pini*, one must wonder if it is susceptible to other pathogens that currently infect *P. nigra*, specifically Sphaeropsis tip blight. Seed source studies may also provide an avenue of research with *P. leucodermis*; however, due to the limited native range of the species, genetic variability within the species may be limited.

Acknowledgements

Thank you is never received quite as seriously as it is given. I have many people whom I owe a sincere debt of gratitude during my career as a graduate student.

Easily, the first people to thank are my co-major professors Drs. Jan Thompson and Jeff Iles for their guidance and support. I sincerely appreciate your assistance not only during the course of the research but in my life. No student can ask for better people to call major professors. I must also thank Dr. Ursula Schuch for her original concept of this project and seeing me as someone who could pull it off. I would also like to thank Drs. Bill Graves and Mark Gleason for serving on my committee and their generous contributions of input. At this time I must also acknowledge Dr. Karen Schaffer and Drs. Johanne and Mahlon Fairchild at Northwest Missouri State University for their friendship, support, and preparation before and during my time here.

For those who assisted me on the "technical" side of research a huge thanks! To Mark Kroggel, Chris Feeley, Stephen Wegulo, Paula Flynn, and Anna Behm for their assistance in the lab. John Smith, Valasia Iakovoglou, Dan Anderson, Katherine Thompson, Danny Prazak, Nathan Kratsch, and Chris Feeley for their skills in field planting and data taking at Reactor Woods. Thank you to those who assisted in harvesting Nate Gassman, Jaclyn O'Grady, and Tammi Skadeland. I owe thanks to Dr. Phillip Dixon and Cory Heilmann for their assistance on the statistics. And of course I have to thank Will Emly, Lynn Shroeder, and the wonderful secretaries in both horticulture and forestry departments for all their assistance.

My fellow graduate students in both horticulture and forestry, thank you for your time and friendship. I have learned so much from all of you. To my wiser and older counterparts Michael Dosmann, Chris Feeley, Jillene Summers, Tom Schultz, Jim Schrader and Sarah Gross—I can't say enough to you in thanks. You helped me through the hardest year of my life with laughter and guidance that I will never forget. To my closest friends in the outside world: Laura, Kyle, Jenny, Matt, Katie, Rob, Loren, Megan, Beth and Seth--thank you for being there for me when I needed you most. I only hope to be as good of a friend to you as you are to me. I owe my sanity to you!

And finally, the people I am thankful for every day—my family. This thesis is dedicated to my father—I know I majored in "brush and weeds" but I hope that you are proud of me. I think of you everyday and miss you terribly. This is also dedicated to Mom, Grandma, and Kristi the three strongest and most understanding women I know. Thank you for not only understanding why I could not always be with you, but why I had to be away. Absolutely none of this would be possible without you. Thank you so very, very much.